Supplementary materials for "A fluorescent, [^{18}F]-positron-emitting agent for imaging PMSA allows genetic reporting in adoptively-transferred, genetically-modified cells"

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Supplementary materials and methods

Cell culture

Human prostate cancer cells (PC3 and PC3-PIP) and human thyroid cancer cells (8505C) were transduced with lentivirus to express luciferase and GFP (Biosettia, San Diego, CA) and PCR tested for mycoplasma. 8505C, PC3, HEK293, and LNCaP cell lines were purchased from ATCC, and PC3-PIP cells were obtained as a gift from Memorial Sloan Kettering Cancer Center (MSKCC). Cells (8505C, PC3, PC3-PIP, and LNCaP) were cultured in RPMI 1640 medium (Corning, USA) and HEK293 cells were cultured in ATCC-formulated EMEM (Corning, USA), supplemented with 10% fetal bovine serum (FBS, Seradigm, USA) and 100 U/mL penicillin/streptomycin (Gibco, USA) at 37 °C in a humidified incubator.

Construction of the PSMA reporter gene

Homo sapien folate hydrolase 1 (*FOLH1*), type II transmembrane glycoprotein (PSMA transcript variant 1, NM_004476.2 (GenBank)) encoding mRNA was harvested from human prostate cancer LNCaP cells. PCR amplification and standard cloning techniques were used to generate dsDNA from the complete mRNA encoding *FOLH1*. dsDNA was subcloned into a pLenti backbone using Xba1 and Sal1 restriction sites.

Lentivirus production and transduction of 8505C cells

We produced lentivirus particles by transiently transfecting HEK293 cells using calcium phosphate.[1] 10 µg of transfer gene (pLenti-PSMA, cloning explained above), 7.5 µg CMV-dR8.2 (Addgene, #8455), and 5 µg pCMV-VSVG (Addgene, #8454) were mixed and incubated with 2 M $CaCl_2$ followed by dilution with 2 x Hanks' Balanced Salt Solution (HBSS). The resulting solution was added dropwise to 10 cm^2 cell culture dishes that had been seeded with 3.2×10^6 HEK293 cells in 10 mL DMEM 24 h earlier. Transfection media was replaced after 6 h. Media containing lentivirus were harvested at 48 or 72 h post transfection, filtered through 0.45 µm filters, and concentrated by ultracentrifugation at 75,000x g for 2 h at 4 °C. The lentivirus was then resuspended in culture medium at an approximate titer of 10^8 IFU/ml. 8505C parental cells (ATCC) were transduced by overnight incubation of lentivirus.[1] Following transduction for 48 h, 8505C cells were tested for expression of PSMA via flow cytometry using APC anti-human PSMA (*FOLH1*) Antibody (Biolegend, clone LNI-17). To get close to 100% transduced

population, cells were stained and sorted via MACS column separation (Miltenyi Biotech, Cambridge, MA) using Anti-FOLH1 antibody and Anti-Mouse MACS beads (Miltenyi Biotech, Cambridge, MA). Cells were sorted by the Weill Cornell Medicine Flow Cytometry Core Facility with a BD Influx sorter.

Verification of PSMA expression

A 1.5×10^5 quantity of PC3, PC3-PIP, 8505C−, and 8505C+ cells were incubated with a human monoclonal PSMA antibody (J591; 1 : 500 in PBS, gift from Dr. Neil Bander, Weill Cornell Medicine) on ice for 30 min. Bound primary antibody was labeled with an APC-conjugated secondary mouse Anti-human antibody (1 : 500 in PBS, #409306, BioLegend, USA) for 30 min at 4 °C. Cells were rinsed with 200 µL PBS and analyzed by flow cytometry (Gallios, Beckman Coulter, USA). 2×10^4 cells were analyzed in each aliquot using KALUZA software (Beckman Coulter, USA).

Cytotoxicity evaluation

8505C cells, human ovarian cancer cells (A2780), or human breast cancer cells (MDA-MB-231) were seeded into 96-well plates at a density of 4,000 cells/well and cultured overnight for adhesion. Different concentrations of ACUPA-Cy3-BF3 ranging from 0.1 nM to 50 µM were co-incubated with cells for 72 h at 37 °C. Cells without ACUPA-Cy3-BF3 treatment were used as controls. Following incubation, cells were rinsed with PBS before being incubated with medium containing 10 µL MTS reagent (Promega, G3581, WI, USA) for 1.5 h at 37 °C. The absorbance of medium was measured at 490 nm by a microplate reader (Infinite M1000 Pro, Tecan, USA).

Confocal microscopy analyses

PC3, PC3-PIP, 8505C− or 8505C+ cells were seeded in an 8-well iBidi µ-slide chamber (iBidi GmbH, Martinsried, Germany) at a density of 2×10^4 cells/well and incubated overnight to achieve adhesion. Cells were incubated with 100 nM of ACUPA-Cy3-BF3 and/or 100 nM LysoTracker™ Deep Red (Catalog: L12492, Thermo Fisher Scientific, USA) for 30 min. Cells were then rinsed with PBS and stained with Hoechst (0.67 µg/mL, Sigma-Aldrich, USA). Cells were then washed with PBS and fluorescent images were taken using a Zeiss LSM 880 confocal laser scanning microscope (Carl Zeiss, Oberkochen, Germany).

Competition experiment

A 1×10^5 quantity of PC3-PIP or PC3 cells were added per well in a 12-well plate and grown overnight. Cells were treated with 100 µM of non-fluorescent, non-radioactive PSMA inhibitor (N-[[[(1S)-5-Amino-1-carboxypentyl]amino]carbonyl]-L-glutamic acid (ACUPA, CAS No. 1025796-32-0)) for 30 min at 37 °C. The mixture was then incubated with 1 µM of ACUPA-Cy3-BF3 in 600 µL culture medium. Cy3.18.OH (1 µM, lacking ACUPA) was used as a non-specific binding control. After 30 min, cells were washed twice with PBS and analyzed by flow cytometry. Epifluorescent microscopy imaging was performed by staining cells with Hoechst in 600 µL PBS for 2 min at room temperature, rinsed twice with PBS, and imaged with EVOS epifluorescence microscope (Life Technologies, USA).

***In vivo* data collection**

Male hairless SCID mice (5-6 week old, 20–25 g) were purchased from Charles River Laboratories (Wilmington, MA, USA). All procedures were approved by the Weill Cornell Medical Center Institutional Animal Care and Use Committee (No. 2014-0030) and were consistent with the recommendations of the American Veterinary Medical Association and the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines.

Subcutaneous implantation of mouse xenograft

Male hairless, severe combined immunodeficient (SCID) mice (5-6 week old, 20–25 g) were purchased from Charles River Laboratories (Wilmington, MA, USA). Mice (n = 8) were anesthetized with 2-3% isoflurane. A 2 million count quantity of PC3 or PC3-PIP cells (luciferase/GFP transduced) in 100 µL PBS/matrigel (1:1, v/v, Catalog 354234, Corning) were subcutaneously implanted into the left and right flanks, respectively, in the upper quadrants. Tumor growth was monitored by bioluminescence imaging weekly by intraperitoneally injecting 3 mg/mL of D-luciferin (50 µL) into anesthetized mice and imaged on a Bruker In-Vivo Xtreme imaging system.

‘Mosaic’ mouse xenograft

8505C+ cells were mixed with increasing 8505C− cells to generate defined mixtures of 8505C+/8505C− cells ranging from 0% to 100% 8505C+ cells. For each subcutaneous tumor, a total of 1 million cells were resuspended in matrigel matrix and xenografted in the flank of male hairless SCID mice (n = 3). The 8505C+ percentages xenografted are 0, 0.1, 1, 10, and 100%, respectively. At 1, 2, and 4 weeks post implantation, bioluminescence imaging was performed by intraperitoneally injecting D-luciferin and imaged on a Bruker In-Vivo Xtreme imaging system.

Intravenous injection of mouse xenograft

Non-obese diabetic (NOD)/SCID gamma (NSG) male mice (5–6 week old) were purchased from Jackson Laboratories. Mice (n = 12) were anesthetized with 2–3% isoflurane, and 0.5 million 8505C− or 8505C+ cells in 100 µL PBS were intravenously injected into the mice through the tail vein. Bioluminescence imaging of tumor growth was performed at 2 and 4 weeks post 8505C cell-injection.

PET/CT imaging

PET images were acquired by injecting 100 µCi of radiolabeled [^{18}F]-ACUPA-Cy3-BF3 (100 µL, 25 µM)[2] by intravenous tail vein injection. Anesthetized mice were fixed on trays in a micro-PET/CT scanner (Inveon, Siemens) and scanned for 10 min (CT) and 15–30 min (PET). Scans were performed 2 h and/or 6 h post-injection. PET/CT scans were performed weekly. The final time-point scan (4 weeks post implantation), mice were injected with [^{18}F]-ACUPA-Cy3-BF3 and then sacrificed by cervical dislocation at 2 h (n = 3) and 6 h (n = 3) post-injection. Gamma scintillated biodistribution was performed at 2.5 or 6.5 h post-injection. PET and CT data were processed using AMIDE v 1.0.4 software and INVEON RESEARCH WORKPLACE software.

***Ex vivo* tissue analysis**

Mice were sacrificed by cervical dislocation at 2–48 h post-injection. Fluorescent imaging studies were performed on whole mice post-mortem. Following fluorescent imaging, selected organs were collected, weighed, and analyzed by scintigraphy, fluorescent imaging, and histopathology. Tissues were transferred to test tubes and counted by scintillated biodistribution on a Wallac Wizard 3.0 gamma counter. Organs were transferred to a Petri dish and fluorescently imaged (exposure = 20 s) using a Bruker In-Vivo Xtreme imaging. Excitation and

emission wavelengths were set at 550 nm/600 nm for ACUPA-Cy3-BF3 and 480 nm/535 nm for GFP. Fluorescent image acquisition and processing were performed using Bruker molecular imaging software. Experiments were repeated a minimum of 3 times per cohort. Following scintillation, tissues were fixed with 4% paraformaldehyde (PFA) in PBS for 4–6 h for fluorescent histological analysis.

Fluorescence-guided surgery and survival studies

A 2 million cell count of 8505C+ or 8505C− cells in 100 µL PBS/matrigel were subcutaneously injected into the left and right flanks, respectively, of male hairless SCID mice (n = 5). Tumors were allowed to grow for 1 month. ACUPA-Cy3-BF3 (7.5 nmols, 50 µM) was intravenously injected into mice 2–48 h prior to surgical tumor resection. Animals were anesthetized with an intraperitoneal injection of a combination of ketamine/xylazine. The anesthetized mice were placed in a prone position and 1.5-2.0 cm lateral incisions were made into overlying skin. Skin was retracted to fully expose subcutaneous tumors. Animals were transferred to the Bruker In-Vivo Xtreme imaging machine and fluorescently imaged. Both lateral tumors (8505C− and 8505C+; or two 8505C+) were carefully removed from mice. 8505C+ resection surgeries were performed alternatively under white light or light with excitation/emission wavelengths of 550 nm/600 nm for ACUPA-Cy3-BF3 and 480 nm/535 nm for GFP. Sutures (4/0, Henry Schein, OH, USA) were placed along the lines of lateral incision. Excised tumor and tissue were collected and fixed in 4% PFA in PBS for subsequent fluorescent histological analysis. The 8505C− tumor was processed as a control. For mice bearing 8505C+ tumor on both flanks, positive margins (~10% of total tumor estimated by ACUPA-Cy3-BF3 fluorescent area) were deliberately not resected. During recovery, mice received meloxicam (2 mg/kg dose) and were placed in a warm plexiglass chamber until complete recovery from anesthesia. Animals were given analgesics for 72 h post-surgery. Tumor regrowth, due to positive margin, was monitored daily for 2 weeks.

Histology

Subcutaneous tumors fixed with 4% PFA in PBS were embedded and frozen in Optimal Cutting Temperature (OCT) medium (Sakura Tissue-Tec, #4585). Sections (20 µm) were generated on a Bright OTF 5000 cryostat (Bright Instruments, UK). Specimens were mounted in Vectashield

antifade medium with DAPI (Vector Laboratories, USA) and fluorescent images were taken using the EVOS epifluorescence microscope.

Statistical analysis

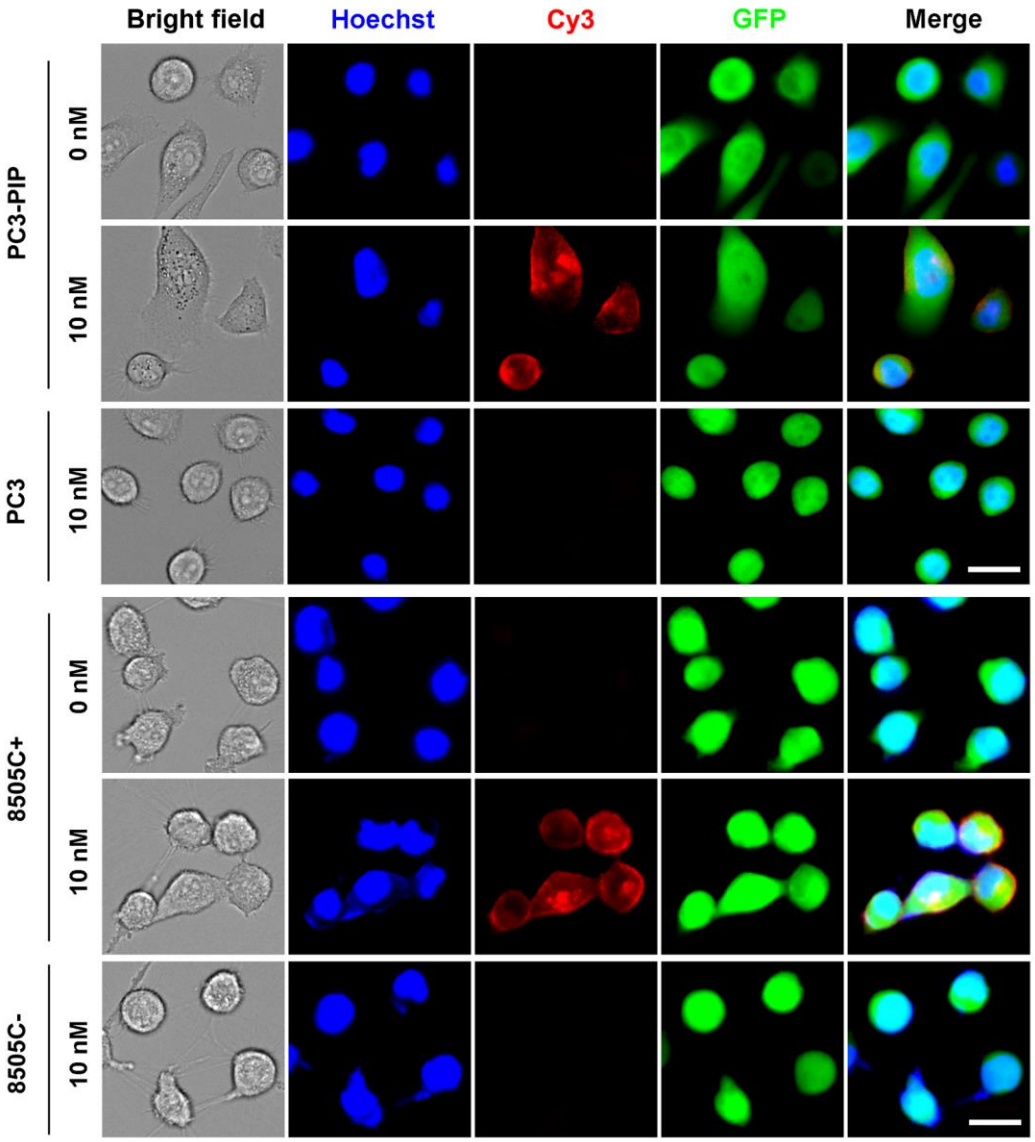
Values are shown as mean ± SD or SEM for all experiments. Data were analyzed and plotted with ORIGIN 7.5 software and ADOBE PHOTOSHOP software. Unpaired 2-tailed student's t-test determined statistical significance of the results and *P* values less than 0.05 were considered significant.

References

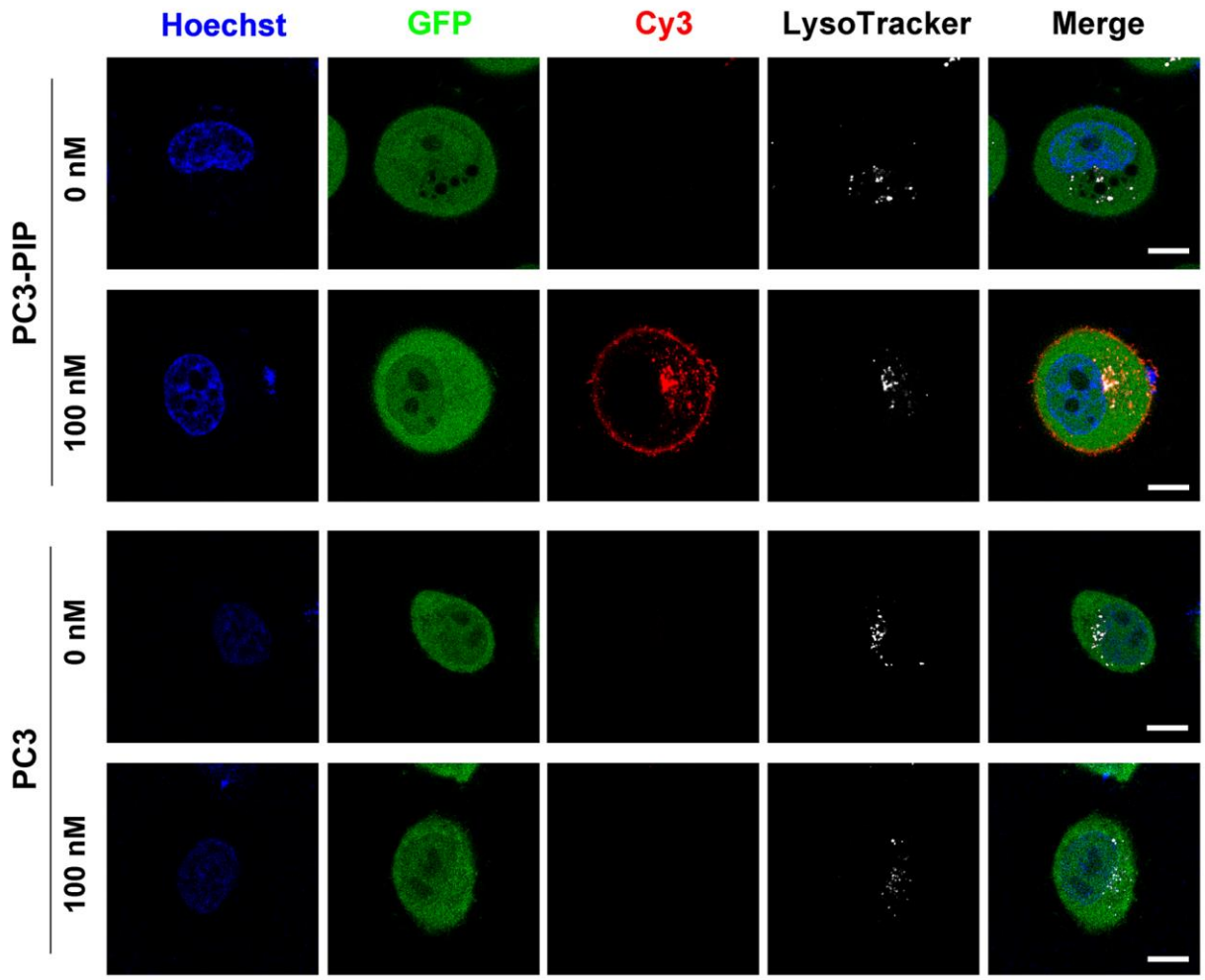
(1) Vedvyas, Y.; Shevlin, E.; Zaman, M.; Min, I. M.; Amor-Coarasa, A.; Park, S.; Park, S.; Kwon, K. W.; Smith, T.; Luo, Y.; Kim, D.; Kim, Y.; Law, B.; Ting, R.; Babich, J.; Jin, M. M. (**2016**) Longitudinal PET imaging demonstrates biphasic CAR T cell responses in survivors. *JCI Insight 1*, e90064.

(2) Kommidi, H.; Guo, H.; Nurili, F.; Vedvyas, Y.; Jin, M. M.; McClure, T. D.; Ehdaie, B.; Sayman, H. B.; Akin, O.; Aras, O.; Ting, R. (**2018**) (18)F-Positron Emitting/Trimethine Cyanine-Fluorescent Contrast for Image-Guided Prostate Cancer Management. *J. Med. Chem. 61*, 4256-4262.

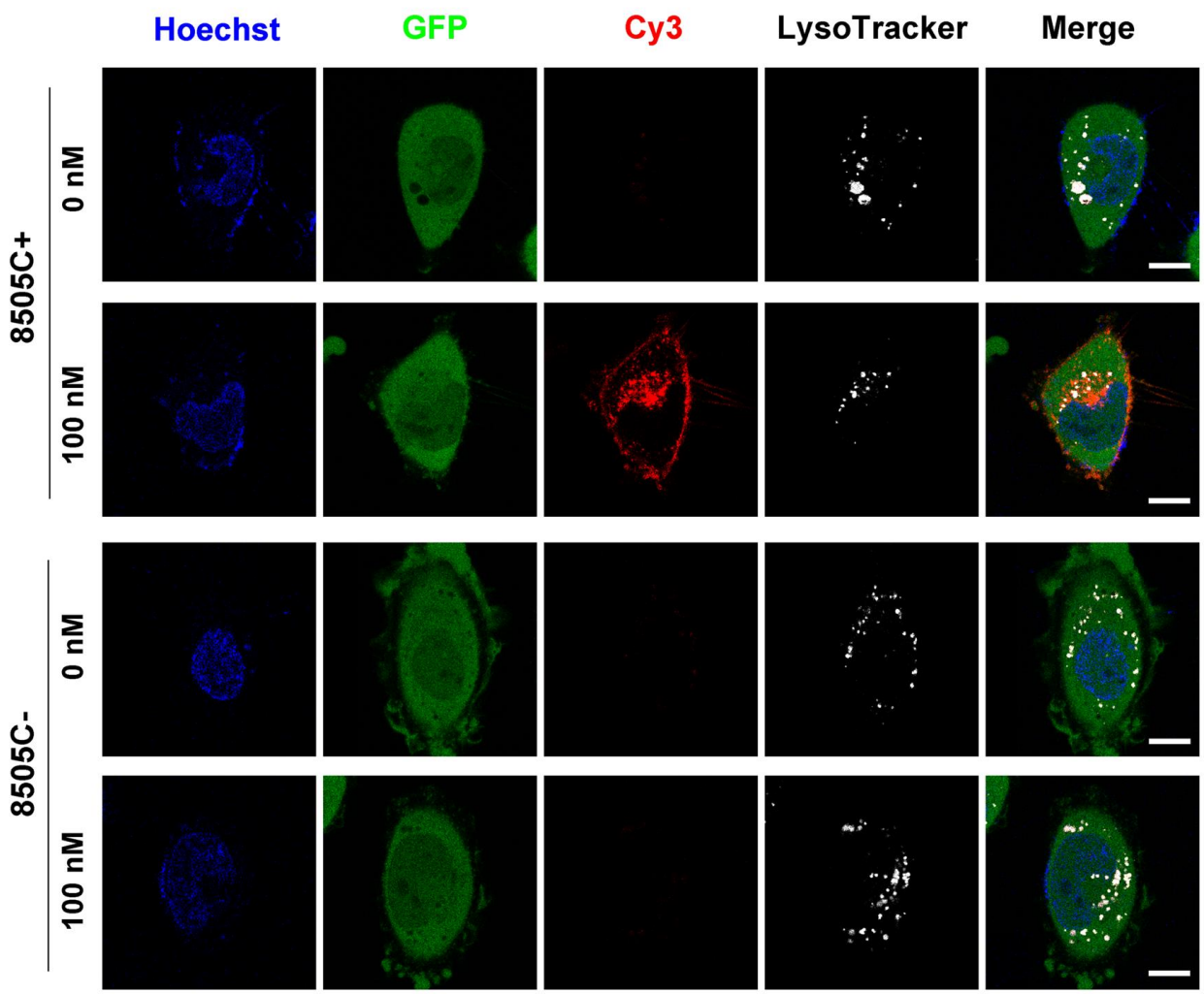
Supplementary Figures



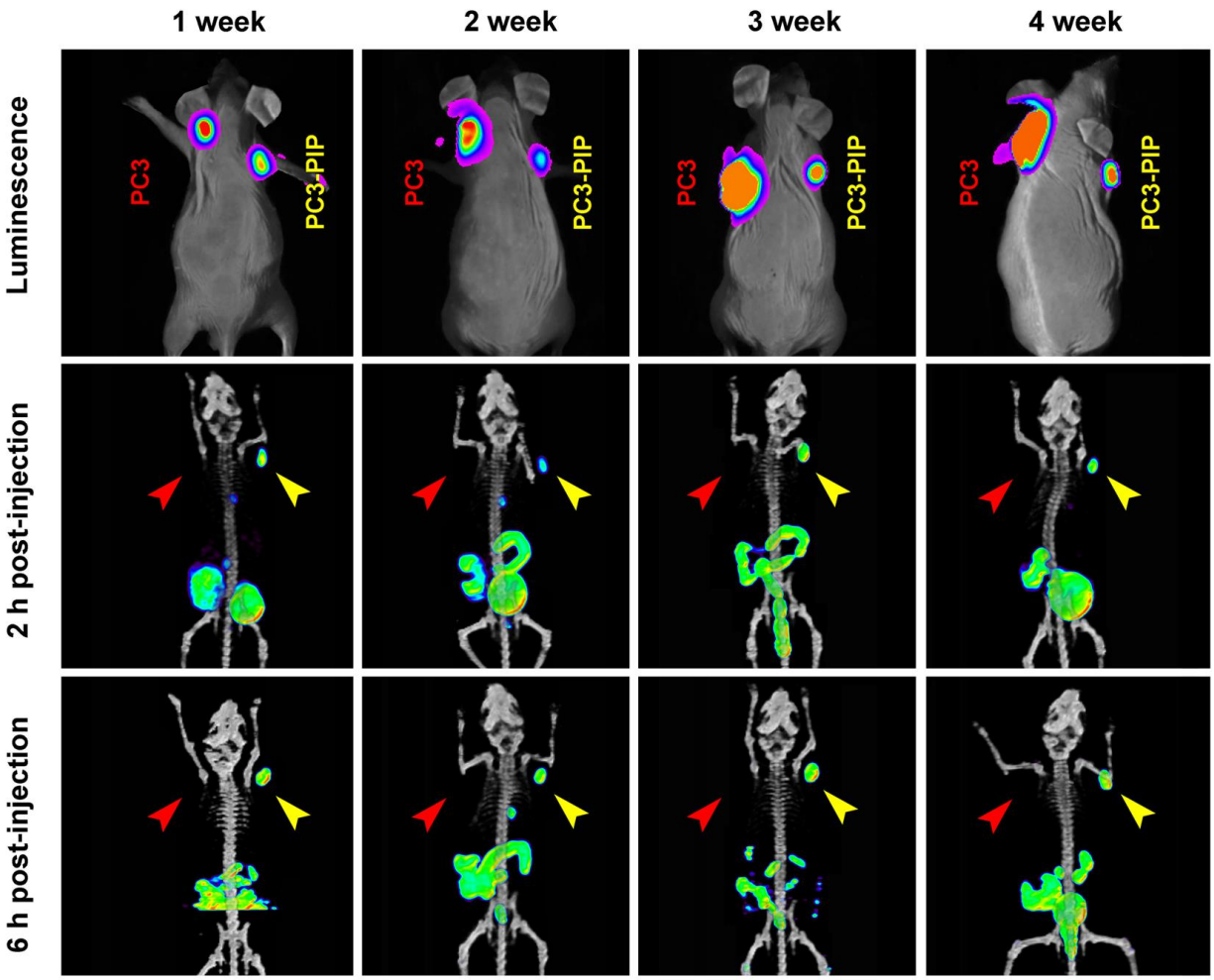
Supplementary Figure 1. Representative epifluorescent images showing ACUPA-Cy3-BF3 affinity to PSMA expressing prostate cancer (PC3-PIP), non-PSMA expressing prostate cancer (PC3), PSMA transduced thyroid cancer (8508C+), and non-PSMA transduced thyroid cancer (8508C−) cell lines. All cell lines were transduced with luciferase and GFP. Results were obtained with an EVOS fluorescent microscope after 1 h incubation of 10 nM ACUPA-Cy3-BF3 with cells. ACUPA-Cy3-BF3 binding was detected with Cy3 fluorescent filters (red, filter at 593/40 nm). Nuclei were imaged with Hoechst staining (blue, filter at 447/60 nm). GFP was visualized using a 510/42 nm filter. Scale bar = 20 µm.



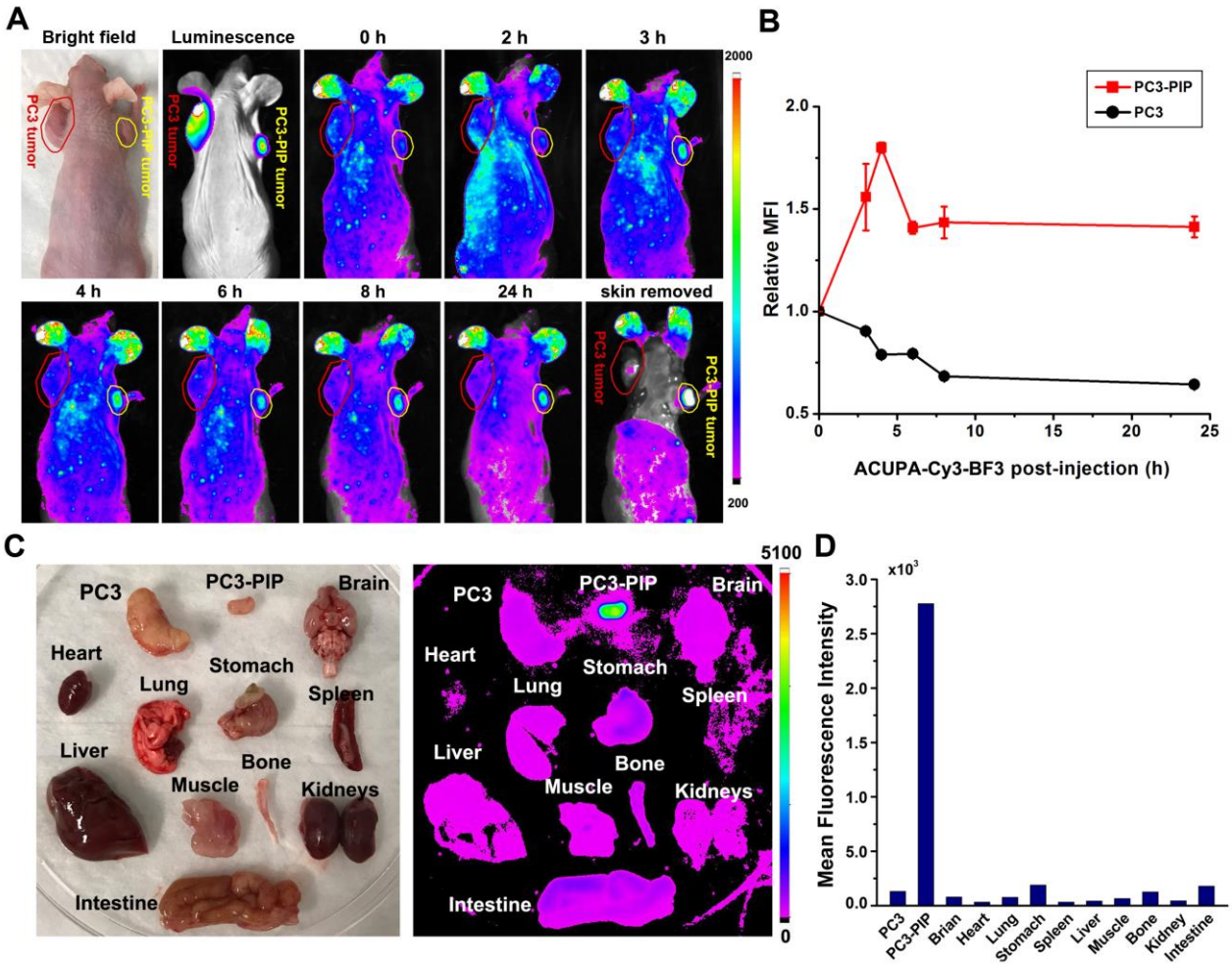
Supplementary Figure 2. ACUPA-Cy3-BF3 localizes to the membrane and internalizes into PSMA-expressing PC3-PIP cells imaged by confocal microscope. PC3-PIP and PC3 cells were treated with 100 nM ACUPA-Cy3-BF3 for 30 min and labeled with 100 nM LysoTracker™ Deep Red (white color, lysosome staining) and 1 mg/mL Hoechst (blue color, nucleus staining). ACUPA-Cy3-BF3 (red) was clearly visible in only PSMA expressing PC3-PIP. ACUPA-Cy3-BF3 binding to the PSMA+ cell membrane and internalization into the PSMA+ cell was observed. Scale bar = 10 μm.



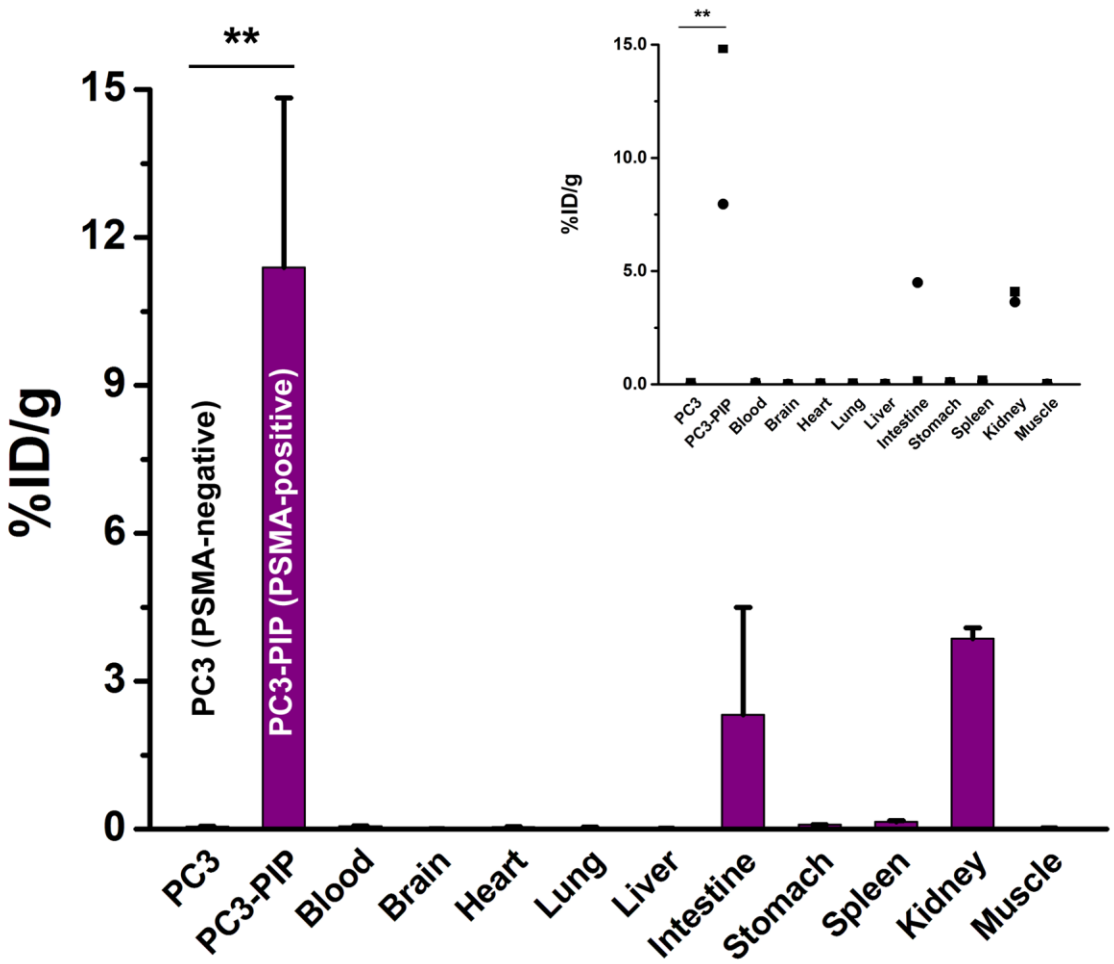
Supplementary Figure 3. ACUPA-Cy3-BF3 localizes to the membrane and internalizes into PSMA-transduced 8505C cells imaged by confocal microscope. Both 8505C− (PSMA-) and 8505C+ cells were treated with 100 nM ACUPA-Cy3-BF3 for 30 min and labeled with 100 nM LysoTracker™ Deep Red (white color, lysosome staining) and 1 mg/mL Hoechst (blue color, nucleus staining). ACUPA-Cy3-BF3 (red) was clearly visible in only PSMA transduced 8505C+. ACUPA-Cy3-BF3 binding to the PSMA+ cell membrane and internalization into the PSMA+ cell was observed. 8505C+ confocal imaging is consistent with the imaging of PC3-PIP cells. Scale = 10 µm.



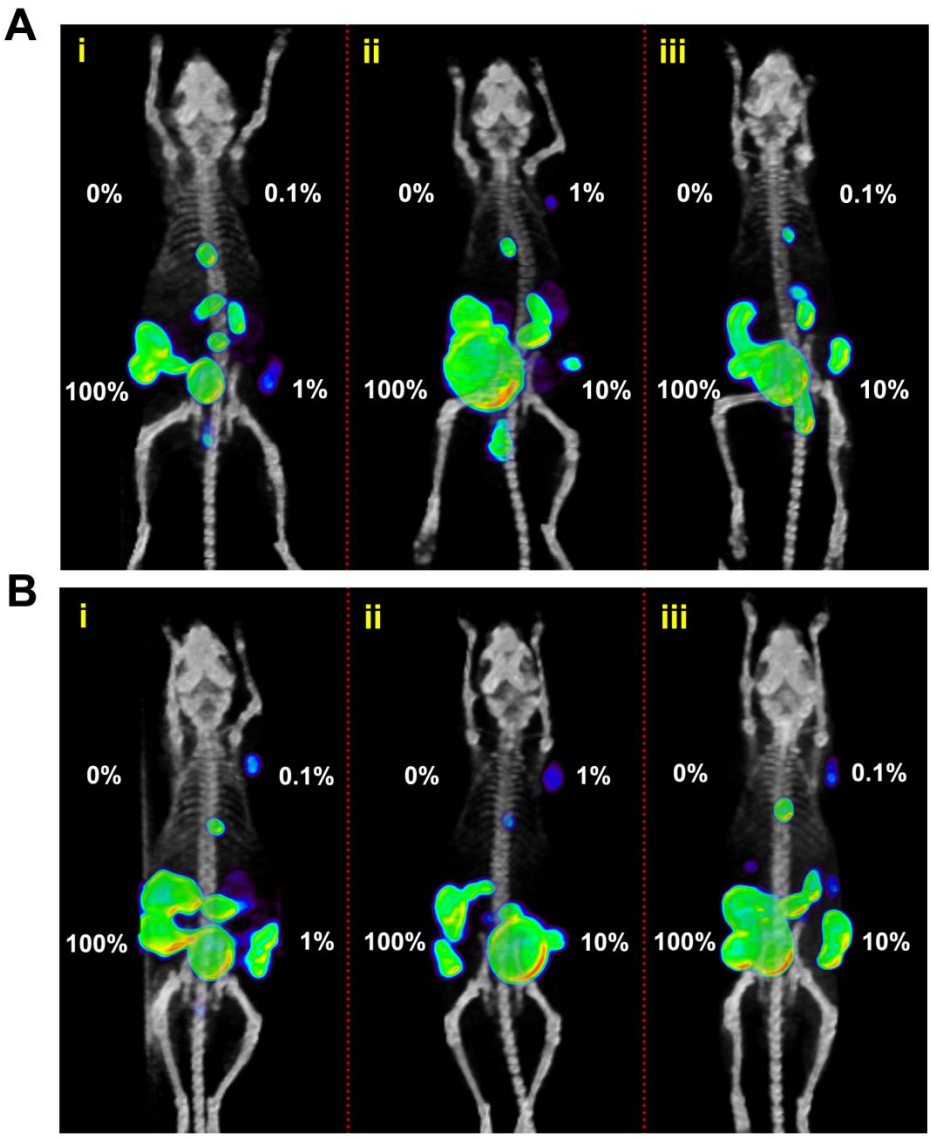
Supplementary Figure 4. *In vivo* bioluminescent and [^{18}F]-ACUPA-Cy3-BF3 PET tracking of PC3 (PSMA−, left flank) and PC3-PIP (PSMA+, right flank) tumorigenesis in a hairless SCID mouse using intravenously introduced [^{18}F]-ACUPA-Cy3-BF3. Representative luminescent (top row) and PET/CT images (middle and bottom rows, maximum intensity projections) of the mouse were acquired at 2 and 6 h post-[^{18}F]-ACUPA-Cy3-BF3 injection (100 µCi, 2.5 nmols) every week following implantation of tumor cells. PC3 tumors grew faster than PC3-PIP tumors. Bioluminescent imaging (top row) was used to track bilateral flank tumor growth. ACUPA-Cy3-BF3 ^{18}F-PET imaging revealed signal only in PSMA expressing PC3-PIP tumors (yellow arrows). Faster growing PC3 tumors (red arrows) in the contralateral flank were not visible by ACUPA-Cy3-BF3 ^{18}F-PET.



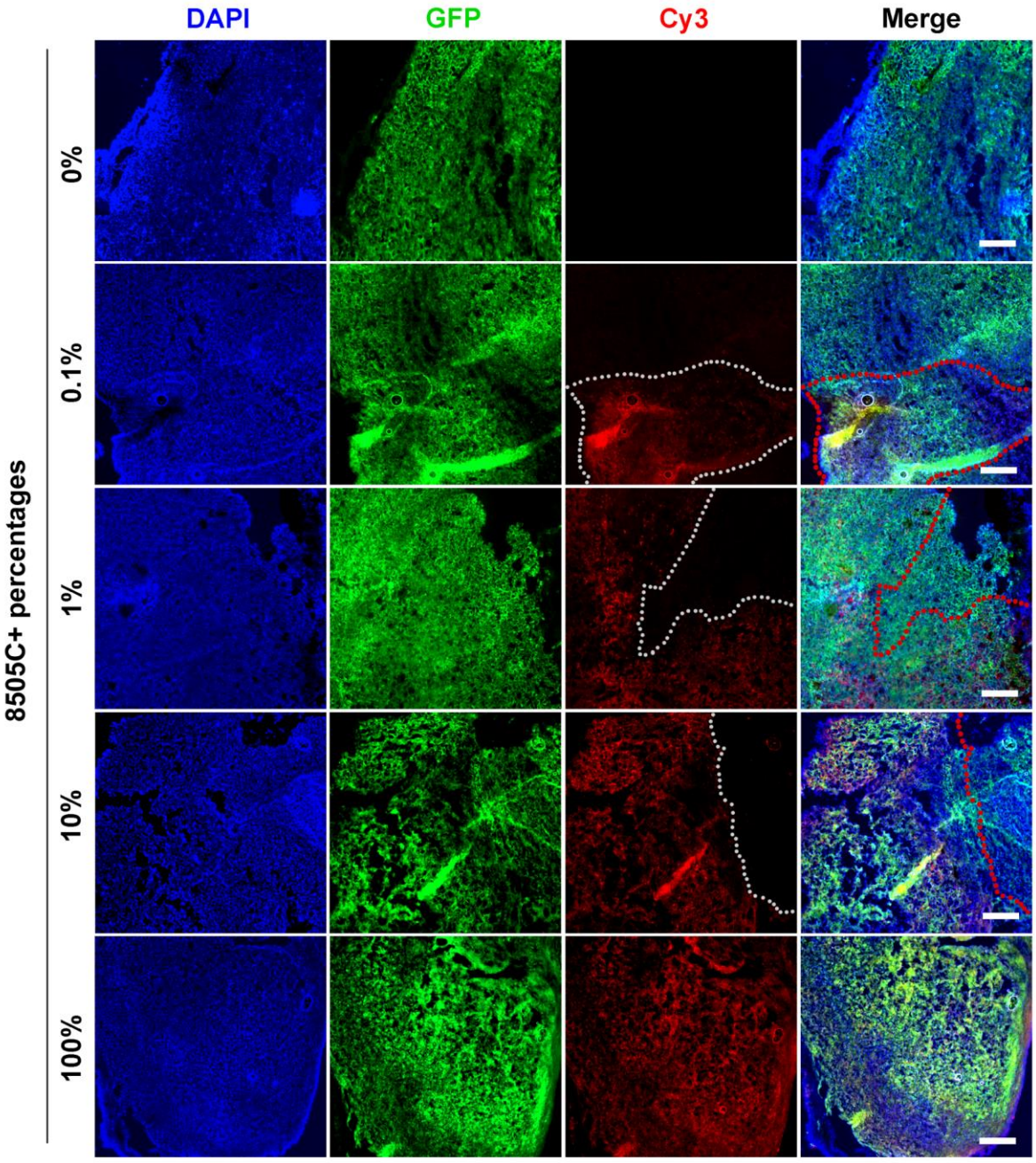
Supplementary Figure 5. *In vivo* and *ex vivo* fluorescent imaging of ACUPA-Cy3-BF3 in a tumor-bearing mouse. Hairless SCID mice were xenografted with PC3 or PC3-PIP cells (2 million cells per site). Tumors were allowed to grow for 1 month. (A) Bright-field, bioluminescence, and ACUPA-Cy3-BF3 fluorescent imaging show ACUPA-Cy3-BF3 on PC3-PIP tumor during 24 h. (B) Quantification of relative mean fluorescent intensity (MFI, relative to 0 hour) at circled areas of interests shown in A. (C) After 24 h tail-vein ACUPA-Cy3-BF3 injection, mice were sacrificed, major organs were collected, and placed on a Petri dish. *Ex vivo* fluorescent imaging was used to confirm tissue biodistribution. (D) Mean fluorescent intensity for different tissues was quantified. ACUPA-Cy3-BF3 was clearly visible in PC3-PIP tissue, with little accumulation in PC3 tumor and healthy tissue.



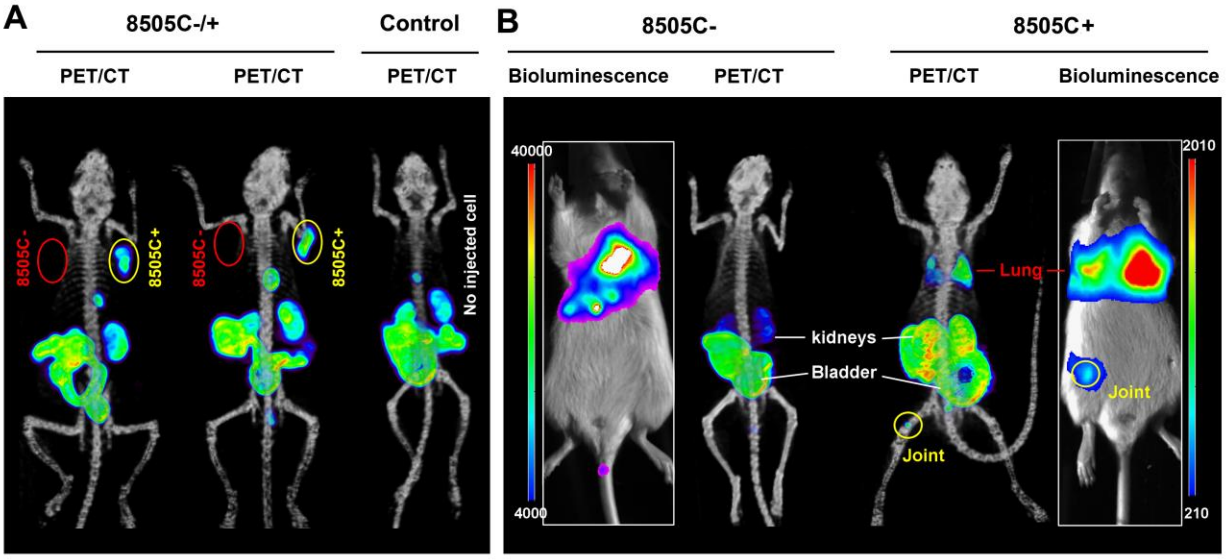
Supplementary Figure 6. Gamma scintillated tissue biodistribution of [^{18}F]-ACUPA-Cy3-BF3 in mice bearing PC3 and PC3-PIP tumors at 2 h post ACUPA-Cy3-BF3 contrast-injection. Mice (n = 2) were xenografted with PC3 and PC3-PIP cells in their bilateral flanks. At 30 days post implantation, [^{18}F]-ACUPA-Cy3-BF3 was injected intravenously, organs were harvested at 2 h post-injection, weighed, and scintillated (gamma scintillation). [^{18}F]-ACUPA-Cy3-BF3 in PC3-PIP tumor and PC3 tumor was 11.4 ± 3.4 %ID/g and 0.05 ± 0.01 %ID/g, respectively. All data points are detailed in the inset. Error bars are ± SEM and ** is $p < 0.01$.



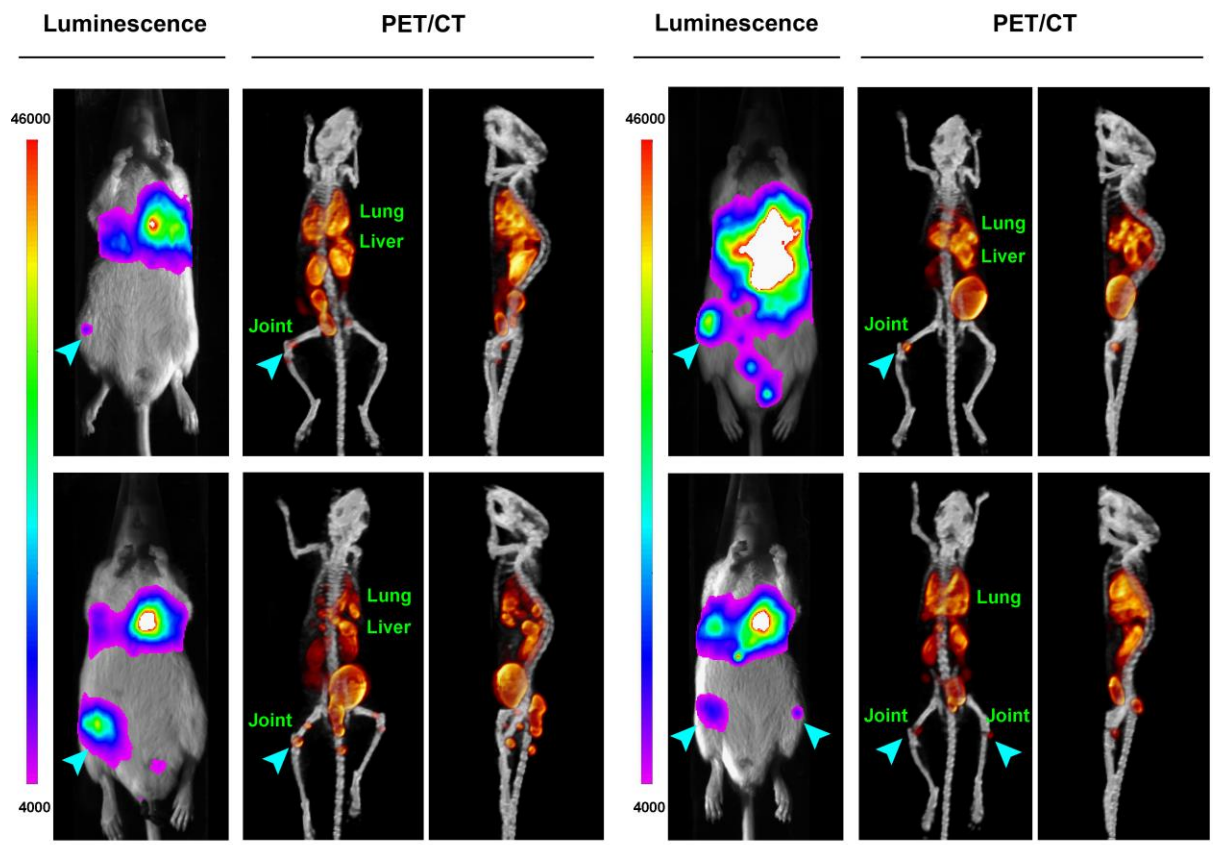
Supplementary Figure 7. [^{18}F]-ACUPA-Cy3-BF3 PET detects dilute, heterogenous PSMA expression in flank tumors of 3 different mice that are labeled (i), (ii), or (iii). 8505C+ and 8505C− cells were mixed and "mosaic" aliquots contained 0, 0.1, 1, 10, and 100% 8505C+ cells. Hairless SCID mice were xenografted with mosaic cell mixtures consisting of 1 million cells in matrigel in 4 quadrants. After 1 week (A) and 2 weeks (B) post implantation, [^{18}F]-ACUPA-Cy3-BF3 was injected via tail vein and PET/CT images were acquired at 2 h post injection. PET signal was visible in "mosaic" tissue containing 8505C+ as low as 1% at 1 week post implantation and 0.1% at 2 week post implantation.



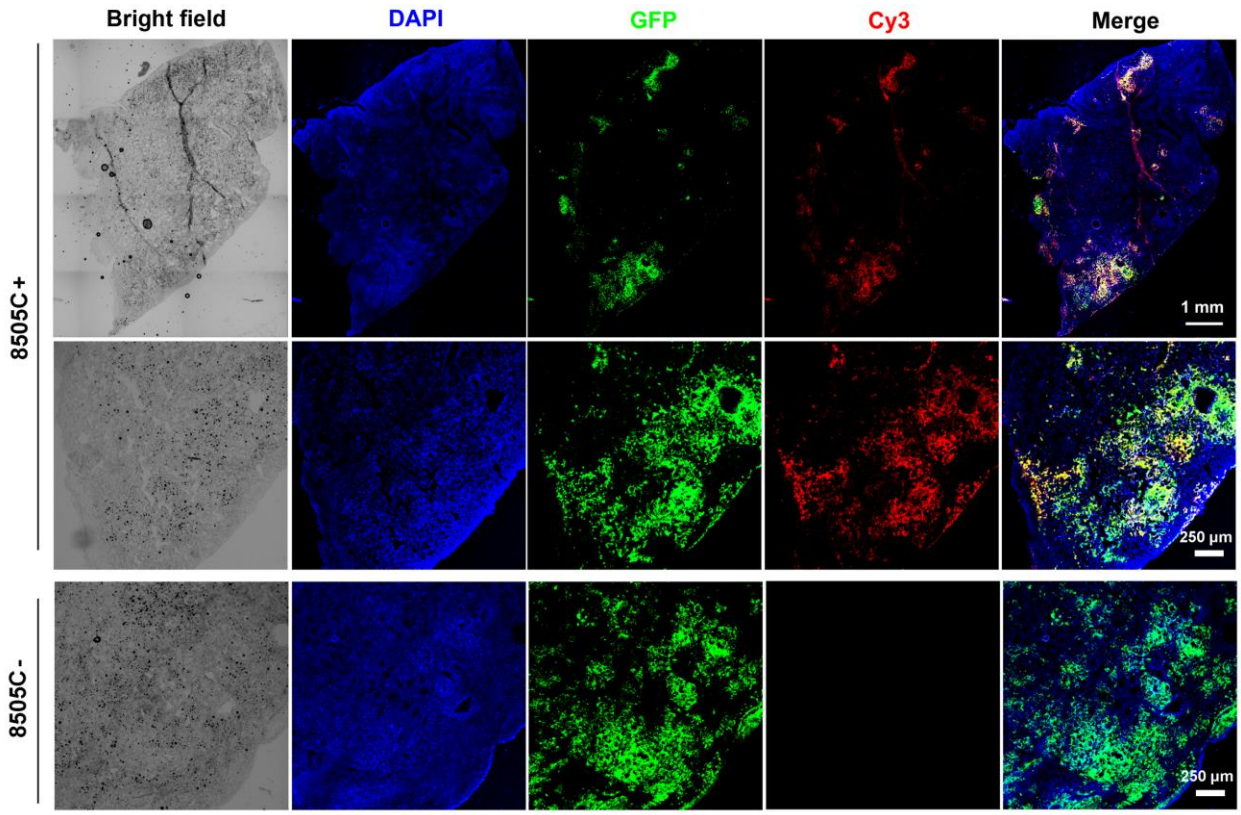
Supplementary Figure 8. Representative fluorescent histology of 'mosaic' tumors containing different percentages of 8505C+ cells. ACUPA-Cy3-BF3 imaging showed heterogeneity in PSMA-expression (Cy3 fluorescence, red). A white line was used to delineate sites of 8505C+ PSMA expression from 8505C- tissue that was visible in GFP imaging. Sections (20 µm) were stained with DAPI (nuclear stain), and imaged on an EVOS epifluorescent microscope. DAPI revealed normal tissue encapsulating a solid 8505C+/8505C- tumor. Scale bar = 250 µm.



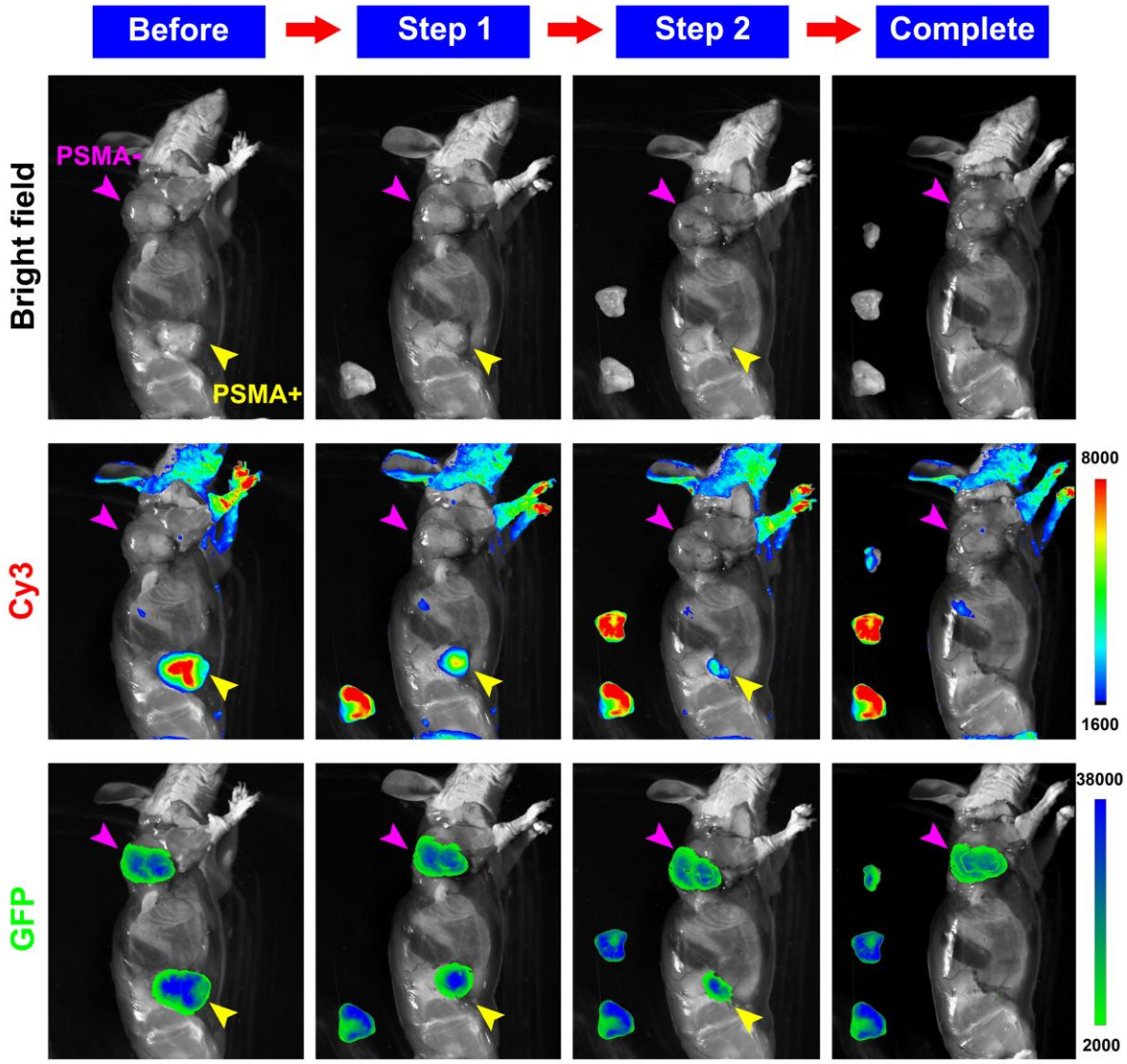
Supplementary Figure 9. HD-GPF imaging in 8505C flank tumors and circulating tumor cell models. (A) 8505C− (3 million cells, left flank) and 3 million 8505C+ (3 million cells, right flank) were xenografted subcutaneously into C57BL/6 mice. PET/CT imaging revealed [^{18}F]-ACUPA-Cy3-BF3 specificity to only 8505C+ flank tissues. A mouse bearing no injected cells was used as a control. (B) An extra-pleural lesion model generated by intravenous injection of 8505C cells into NSG mice. Bioluminescent imaging at 2 weeks post 8505C implantation show 8505C+ and 8505C- localization in the lungs. An [^{18}F]-ACUPA-Cy3-BF3 **(**100 µCi) PET scan performed 2 h post-injection confirmed bioluminescent data. ACUPA-Cy3-BF3 and bioluminescent colocalization in 8505C+ (but not 8505C−) models demonstrate HD-GPF targeting of metastatic lesions in the lung and joint.



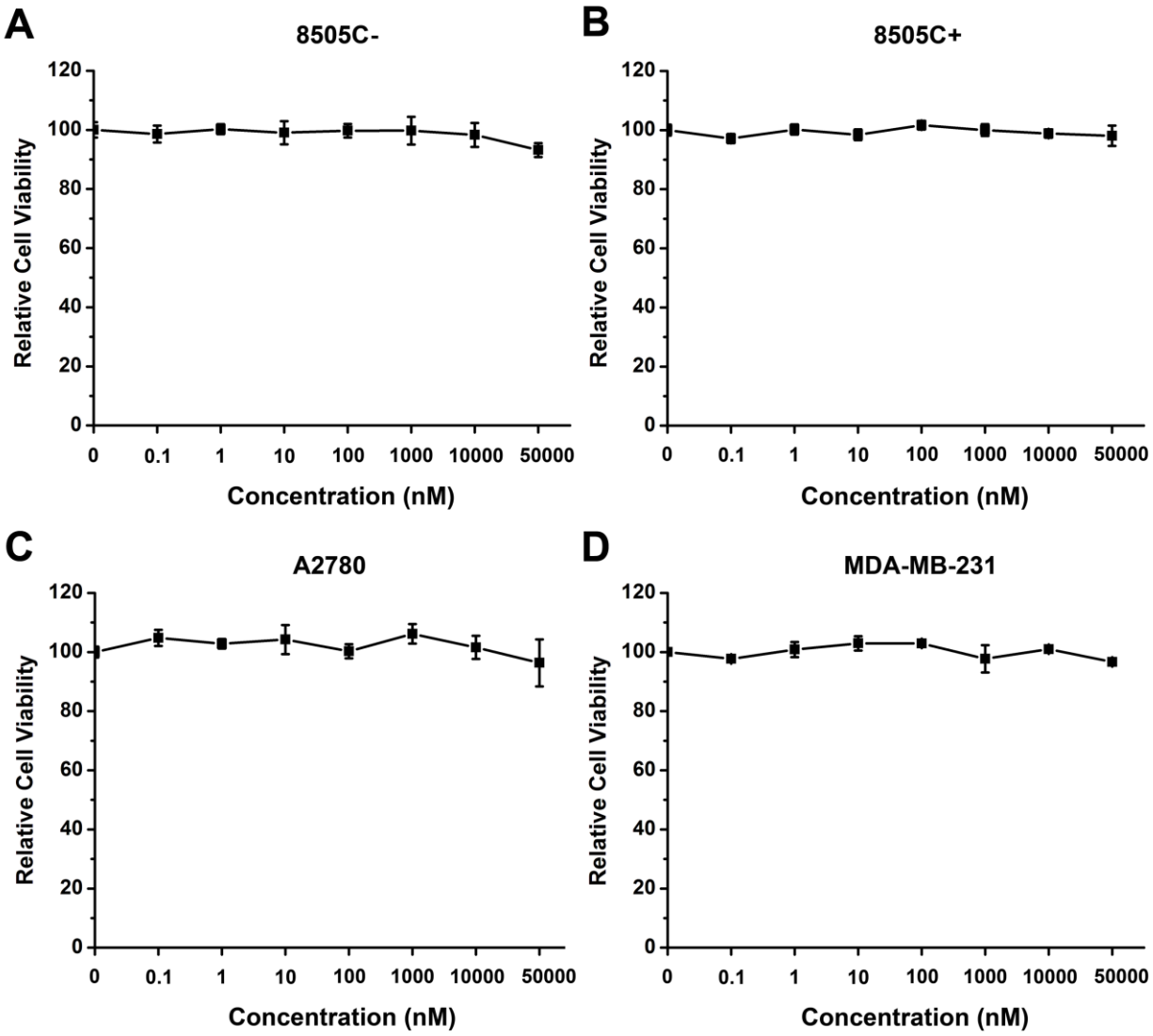
Supplementary Figure 10. Tumor burden was visible in additional NSG mice bearing 8505C+ (0.5 million cells) introduced through the tail vein (n = 4). Tumor burden was visualized by bioluminescence at 4 weeks post injection.



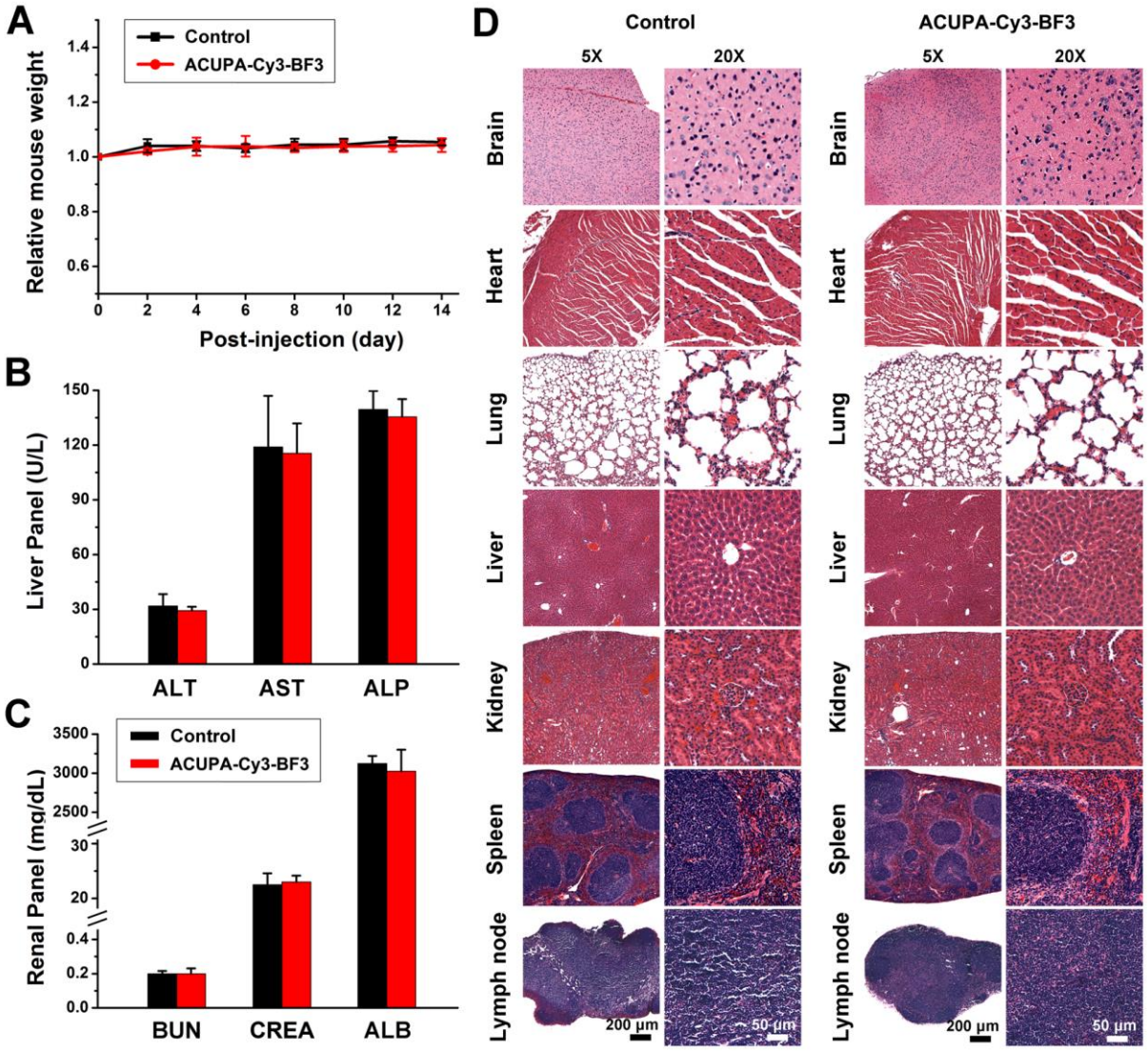
Supplementary Figure 11. Comprehensive imaging of lung tissue of mice shown in Figure 5. Fluorescent GFP histology demonstrated that both 8505C+ and 8505C− cells invaded lung tissue, while ACUPA-Cy3-BF3 fluorescence was only present with injection of 8505C+ cells that express PSMA.



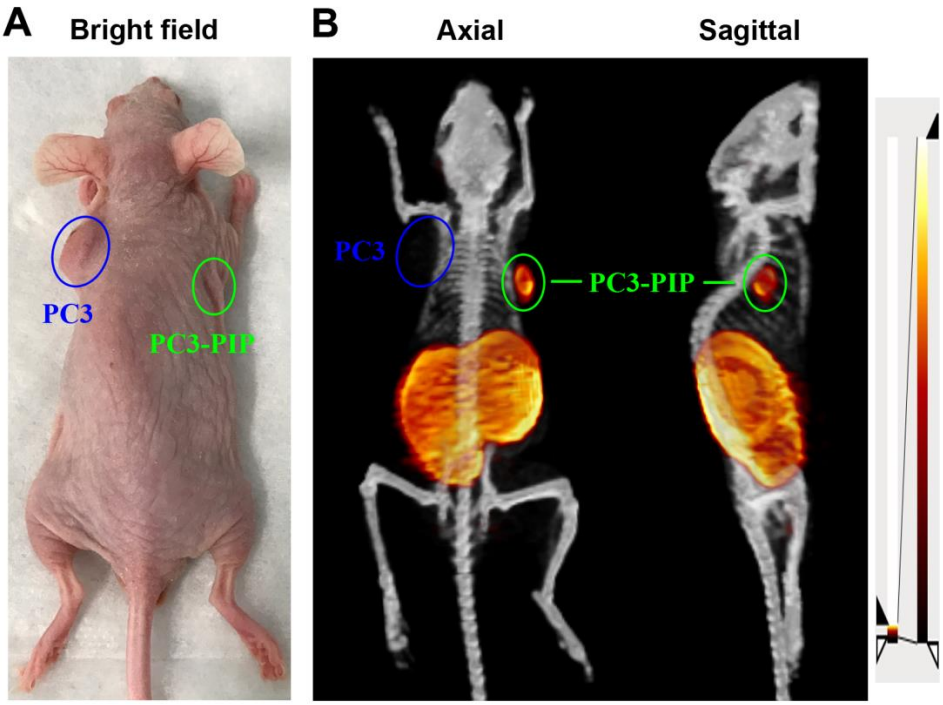
Supplementary Figure 12. Intraoperative ACUPA-Cy3-BF3 fluorescence imaging was able to guide accurate resection of primary tumors in mice. Hairless SCID mice bearing 4 weeks 8505C- (upper torso) and 8505C+ (pelvic area) tumors were intravenously injected with ACUPA-Cy3-BF3 (7.5 nmols). Following 48 h injection, mice were sacrificed and primary tumor resection was attempted. Using ACUPA-Cy3-BF3 fluorescence-guided surgery, three attempts at resection were required to completely remove 8505C+ solid tumor (yellow arrows). Note that the 8505C− tumors (magenta arrows) were visible in GFP channel but not Cy3 channels (ACUPA-Cy3-BF3) intraoperatively. Positive/negative margins were confirmed in both ACUPA-Cy3-BF3 and GFP fluorescent channels. ACUPA-Cy3-BF3 excitation/emission = 550/600 nm; and GFP =480/535 nm.



Supplementary Figure 13. Cytotoxicity of 50 µM ACUPA-Cy3-BF3 was not observed after 72 h incubation on four cell lines. Data were acquired using a CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS, #G3581, Progema, USA) with (**A-B**) human thyroid cancer cells (8505C- and 8505C+), (**C**) human ovarian cancer cells (A2780), and (**D**) human breast cancer cells (MDA-MB-231). The cells were treated with concentrations of 0.1 nM - 50 µM ACUPA-Cy3-BF3 at 37 °C for 72 h. Error bars are ± SD (n = 4).



Supplementary Figure 14. Adverse ACUPA-Cy3-BF3 effects were not observed at 75 nmols intravenous administration. Healthy Balb/c mice were intravenously injected (tail-vein) with a 1x PBS control or 75 nmols (10-fold higher than the imaging dose) of ACUPA-Cy3-BF3 and monitored for 14 days (n = 4). (A) Relative weight of mice after injection. (B) Clinical serum chemistry of liver panel in each group of mice assaying alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activity. (C) Clinical serum chemistry of renal panel in each group of mice, including blood urea nitrogen (BUN), creatinine (CREA), and albumin (ALB). The other serum chemistry parameters are shown in Supplementary Table 1. (D) Representative H&E staining of brain, heart, lung, liver, kidney, spleen, and lymph node sections in each group of mice after paraffin embedding. Error bars are ± SD.



Supplementary Figure 15. A PET scan was performed of a mouse bearing both PC3 and PC3-PIP tumors using ^{68}Ga-HBEDCC. Hairless SCID mice (*n* = 3) were xenografted with 2 million PC3 or PC3-PIP cells in the left and right flanks, respectively, and tumors were allowed to grow for 3 weeks. ^{68}Ga-HBEDCC (100 µCi, gift by Anastasia Nikolopoulou at Weill Cornell Medicine) was injected intravenously through the tail vein of mice. PET/CT was performed at 2 h post-injection. (A) Bright field image showing one mouse bearing PC3 and PC3-PIP tumors on its back. (B) PET/CT (30 min PET collection) of Ga^{68}-HBEDCC axial and sagittal views showed ^{68}Ga-HBEDCC accumulation in only PC3-PIP tumor (1.82 ± 0.78 %ID). ^{68}Ga-HBEDCC signal in kidney was significant higher than ACUPA-Cy3-BF3 shown in supplementary Figure 4.

Supplementary Table 1. Results of serum chemistry tests for mice (n = 4) in Supplementary Figure 14. The control group was 1x PBS and the treated group was 75 nmols ACUPA-Cy3-BF3 (10 fold higher than the imaging dose) intravenous injection. The mice were monitored and weighed for 14 days prior to serum testing.

Chemistry panel	**Control**		**ACUPA-Cy3-BF3**	
	Mean	**SD**	**Mean**	**SD**
BUN (mg/dL)	22.5	2.082	23	1.15
CREA (mg/dL)	0.2	0.016	0.2075	0.03
BUN/CREA ratio	112.9	12.51	112.3	14.35
ALP (U/L)	139.5	10.15	135.5	9.68
ALT (U/L)	31.75	6.551	29.25	2.06
AST (U/L)	119	27.94	115.5	16.34
GGT (U/L)	0	0	0	0.00
TBIL (mg/dL)	0.2	0	0.2	0.00
DBIL (mg/dL)	0	0	0	0.00
IBIL (mg/dL)	0.2	0	0.2	0.00
TP (g/dL)	4.975	0.15	4.85	0.37
ALB (g/dL)	3.125	0.0957	3.025	0.28
GLOB (g/dL)	1.85	0.0577	1.825	0.10
A/G ratio	1.689	0.0234	1.6551	0.07
P (mg/dL)	5.4	0.5354	6.175	0.97
Ca (mg/dL)	8.75	0.2082	8.425	0.57
GLU (mg/dL)	91.25	8.958	106	18.37
CHOL (mg/dL)	81	2.309	76	5.48
TRIG (mg/dL)	112.5	10.08	95.25	22.25
CK (U/L)	1310.5	318.6	1340	257.78
TCO_2 (mEq/L)	16	1.414	14.5	0.58
Na (mEq/L)	160.5	3.873	157.5	7.68
K (mEq/L)	10.35	0.5196	11.65	1.01
Cl (mEq/L)	121.25	2.363	121.25	7.27
Na/K ratio	15.5	0.5774	13.5	0.58
Anion Gap	33.75	2.062	33.5	2.38

BUN: blood urea nitrogen; CREA: creatinine; ALP: alkaline phosphatase; ALT: alanine aminotransferase, AST: aspartate aminotransferase; GGT: gamma glutamyl transferase; TBIL: total bilirubin; DBIL: direct bilirubin; IBIL: indirect bilirubin; TP: total protein; ALB: albumin; GLOB: globulin; A/G: albumin to globulin; GLU: glucose; CHOL: cholesterol, TRIG: triglycerides; CK: creatine kinase; TCO_2: total carbon dioxide.